

Original Article

Serum Neopterin Levels in Children with Primary Nephrotic Syndrome

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ABSTRACT

Background: Neopterin (NP) is a pyrazino-pyrimidine compound derived from guanosine 5'-triphosphate. NP production has been linked to the activation of cell-mediated immunity. Increases in serum NP concentration and urinary NP excretion have been used as diagnostic or prognostic markers in various immune-mediated clinical conditions including allograft rejection, infectious diseases, autoimmune diseases, and neoplastic diseases.

Objectives: To assess the changes that occur in serum neopterin levels in children with primary nephrotic syndrome (PNS).

Methods: Serum NP levels were measured by ELISA in 38 children with active PNS (27 males, 11 females; aged 6.4 ± 2.9 years) (Group I) and 17 children (11 males, 6 females; aged 7 ± 2.5 years) with PNS in remission (Group II) and 20 age- and sex-matched controls. All patients had normal creatinine clearance. Among Group I children, 28 patients were steroid-sensitive (SSNS) while 10 patients were steroid-resistant (SRNS).

Results: Serum NP levels were significantly elevated in Group I patients (median = 7.5, range 1.8-10.8 ng/ml) compared to Group II (median = 1.5, range 0.5-2.5 ng/ml, $p < 0.001$) and controls (median = 0.6, range 0.1-2.7 ng/ml, $p < 0.001$). Group II patients had similar NP levels compared to controls ($p = 0.71$). There was a significant positive correlation between serum NP levels and the degree of proteinuria in Group I patients ($r = 0.4$, $p = 0.01$). No significant differences in serum NP levels were noted between SSNS and SRNS patients ($p = 0.4$).

Conclusions: Serum NP could be used as a marker of the activity of PNS but it could not be used to differentiate between SSNS and SRNS.

INTRODUCTION

The association of cellular immune disorders with the pathogenesis of nephrotic syndrome has been proposed in minimal change disease and in other types of glomerulonephritis. Thus, patients with nephrotic syndrome may share cell-mediated immune disorders contributing to increased glomerular permeability irrespective of the specific glomerular manifestations^(1,2).

Neopterin (NP) is a pyrazino-pyrimidine compound derived from guanosine 5'-triphosphate. NP production has

been linked to the activation of cell-mediated immunity⁽³⁾. Increases in serum NP concentration and urinary NP excretion have been used as diagnostic or prognostic markers in various immune-mediated clinical conditions including allograft rejection, infectious diseases, autoimmune diseases, and neoplastic diseases^(4,5).

AIM OF THE WORK

This study was done to evaluate the changes that occur in serum NP levels in children with PNS.

SUBJECTS AND METHODS

Patients

This study was conducted on 38 children with active PNS (Group I) and 17 children with PNS in remission (Group II). Out of Group I patients, 28 children had steroid sensitive nephrotic syndrome (SSNS) while 10 patients had steroid resistant nephrotic syndrome (SRNS). Patients were recruited consecutively from the Pediatric Nephrology Unit, Mansoura University Children's Hospital, during the period from December 2000 to November 2001. Informed consent was taken from the parents. Patients were diagnosed according to the criteria submitted by International Study of Kidney Disease in Children⁽⁶⁾.

Since under normal renal function serum NP concentration reflects the status of cellular immunity⁽⁷⁾, the study was restricted to patients with normal renal functions. Patients who have conditions that may affect NP production as infection were excluded.

Twenty healthy age- and sex-matched children served as controls. They had no evidence of renal diseases or other conditions that may affect NP production. Clinical and laboratory criteria of the studied groups are summarized in Table 1.

Methods

Sera were collected from Group I patients early in the course of the disease before starting steroid therapy. In Group II, sampling was done at least one month after discontinuation of steroids.

Sera were stored at 20°C until the time of assay. Assay was done by using neopterin enzyme immunoassay (96-kit, IBL GmbH, Hamburg, Germany). The

assay principle follows the basic principle of competitive ELISA: the competition between the neopterin in the sample to be assayed and the enzyme conjugate (neopterin-peroxidase) employed as tracer for binding to antibody immobilized in the wells of a micro titer. The sample and the neopterin-peroxidase conjugated are added to the wells of the micro titer plates where they compete for a number of antibody sites. After incubation wells are rinsed to remove unbound components. Bound enzymatic activity is measured by the addition of a chromogenic substrate. The intensity of color developed is inversely proportional to the concentration of neopterin in the sample.

Statistical analysis

Statistical analysis was done by using SPSS (Statistical Package for Social Sciences) program version 10, 1999. Data had non parametric distribution when tested using Kolmogorov-Smirnov Test. Data were presented in the form of median and range. Mann-Whitney test was used for comparison between the studied groups. To study the correlation between the degree of proteinuria in Group I and NP levels Spearman rank correlation coefficient was used. Significance was considered when p values were less than 0.05.

RESULTS

Table 2 shows that serum NP levels were significantly elevated in Group I patients (median = 7.5, range 1.8-10.8 ng/ml) compared to Group II (median = 1.5, range 0.5-2.5 ng/ml, $p < 0.001$) and controls (median = 0.6, range 0.1-2.7 ng/ml, $p < 0.001$). Group II patients had similar NP

levels compared to controls ($p = 0.71$). There was a significant positive correlation ($r = 0.4$, $p = 0.01$) between serum NP levels and the degree of proteinuria in Group I

patients (Fig. 1). No significant differences in serum NP levels were noted between SSNS and SRNS patients ($p = 0.4$) as shown in Fig. 2.

Table 1: Clinical and laboratory criteria of the studied groups.

	Group I (n = 38)	Group II (n = 17)	Controls (n = 20)
Age (years)	6.4 ± 2.9	7 ± 2.5	5.8 ± 2.2
Sex (males/females)	27/11	11/6	10/10
Hypertension (±)	9/29		
Hematuria (±)	6/32		
24 hour urinary protein (g/m²)	2.6 ± 1.3		
Serum albumin (g/dl)	2.0 ± 0.6		
Serum cholesterol (mg/dl)	396.6 ± 129.8		
Serum creatinine (mg/dl)	0.9 ± 0.2		
Serum C₃ (mg/dl)	148.1 ± 47.0		

Table 2: Comparison of serum neopterin (NP) levels between the studied groups and controls.

Group	n	Serum NP (ng/ml)		p	
		Median	Range	p₁	p₂
Group I	38	7.5	1.8-10.8	< 0.001	< 0.001
Group II	17	1.5	0.5-2.5	0.71	
Controls	20	0.8	0.1-2.7		

p₁ = Group I and II versus controls
p₂ = Group I versus group II

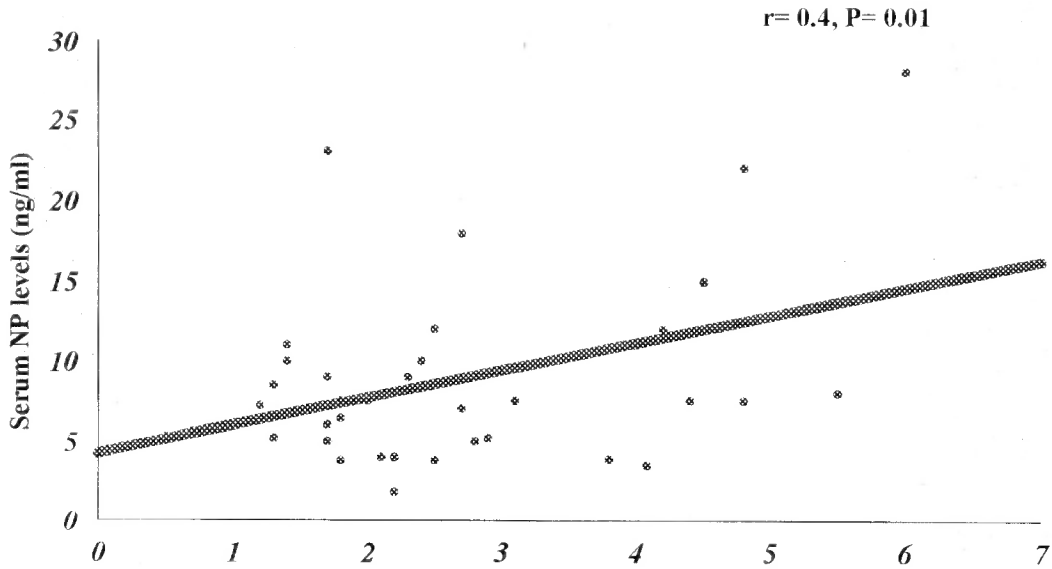


Fig. 1. Correlation between the degree of proteinuria and serum neuplerin levels in group 1 patients

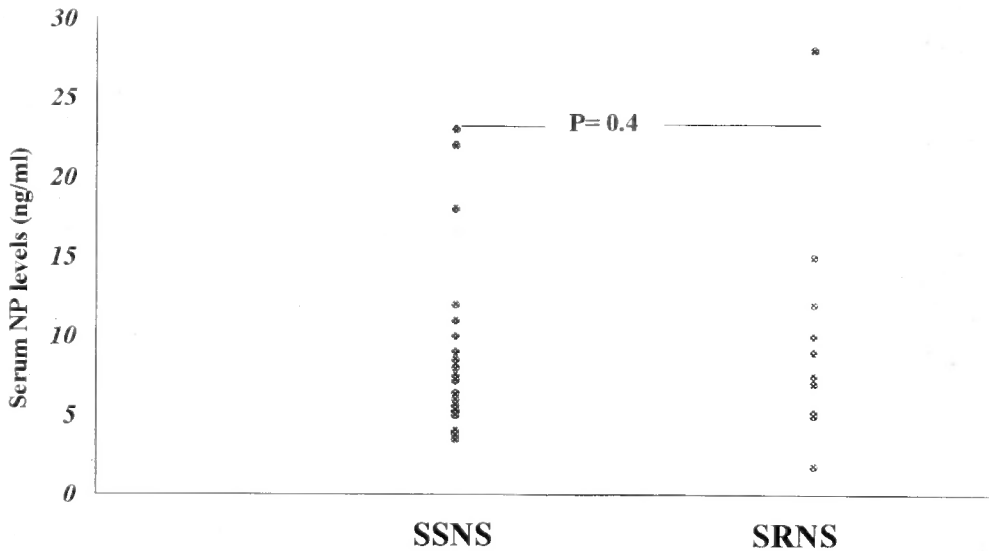


Fig. 2. Comparison of serum neuplerin levels between SSNS and SRNS

DISCUSSION

Our study showed that children with active PNS had elevated serum NP levels compared to controls and remission of the disease was associated with normalization of serum NP.

In agreement with our results, Oda

et al.⁽⁸⁾ proved that adults with PNS, irrespective of the pathohistology of the glomeruli, had elevated serum NP concentrations and urinary NP/Cr ratios compared with healthy controls. They also noticed that remission of the disease was associated with decreased urinary NP/Cr ratios. In patients

with hepatitis B nephropathy, the urinary NP excretions were markedly increased only in the presence of nephrotic syndrome or heavy proteinuria⁽⁹⁾. Also both plasma and urinary NP levels have been increased in aggressive glomerulonephritis⁽¹⁰⁾. Leohiran et al.⁽¹¹⁾ showed that NP concentrations were higher in patients with active lupus nephritis than in patients with inactive disease and healthy controls.

Our study demonstrated the presence of a significant positive correlation between serum NP levels and the degree of proteinuria in children with active PNS. Similarly, Lin⁽⁹⁾ showed that in patients with hepatitis B-nephropathy, urinary NP levels fell as proteinuria decreased. Moreover, serial determinations of NP in active lupus nephritis patients showed a rapid decrease of initially high concentration, paralleling a decline of clinical activity after initiation of steroid therapy⁽¹¹⁾. These findings point to the association between the activity of the nephrotic syndrome and NP levels.

NP is produced by human macrophages on stimulation by gamma interferon⁽³⁾.

Because this lymphokine is released by activated T-lymphocytes NP represents a sensitive marker of T-lymphocytes/macrophage interplay⁽⁷⁾.

In minimal change nephrotic syndrome, various reports have emphasized the contribution of cellular immune disturbances to the nephrotic state⁽¹²⁻¹⁴⁾. Infusion of supernatants from cultures of lymphocytes from patients with minimal change nephrotic syndrome into normal rats causes increased urinary protein excretion^(12,13). Thus the elevated serum NP levels in the studied children may reflect the T-cell dysfunction that is known to occur in these patients.

We also demonstrated no significant differences in serum NP levels between patients with SSNS and SRNS. Another study showed that urinary NP values before steroid therapy were similar between the steroid-responsive and the steroid-resistant patients with nephritic syndrome⁽⁸⁾.

In conclusion, serum NP could be used as a marker of the activity of PNS but it could not be used to differentiate between SSNS and SRNS.

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